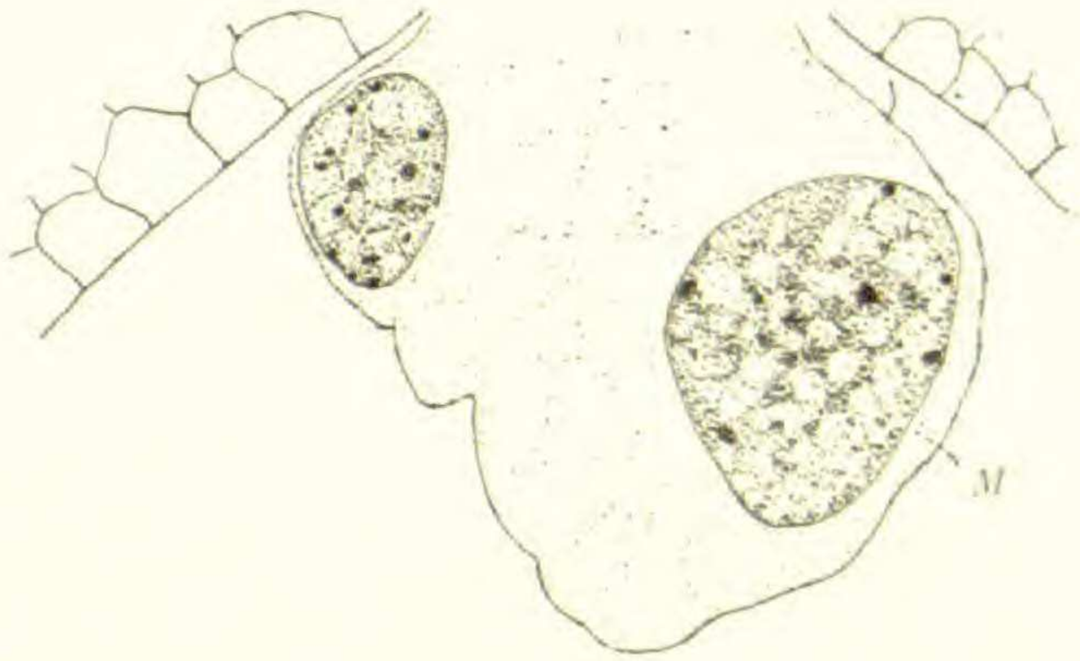
BRIEFER ARTICLES.

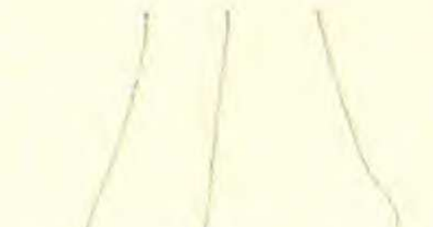
NOTES ON THE FERTILIZATION AND EMBRYOGENY OF CONIFERS.

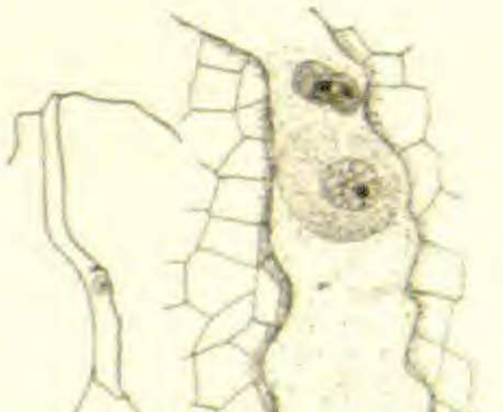
(WITH PLATE VI)

DURING the autumn quarter of 1896 a group of graduate students under my direction made a study of the special morphology of gymnosperms. The necessities of the material restricted critical work to the conifers, and among them *Pinus* and *Taxus* were represented by the most complete series of stages. The problems of special interest were those of fertilization and embryogeny, following such papers as those of Belajeff, Dixon, and others. The work was supplementary to the regular research work among angiosperms in which each student is engaged, and preparations made for classes in elementary morphology were freely used. As a consequence, the material was sometimes in such a condition of staining, etc., that some points of critical interest could not be cleared up by proper technique. The work of the authors referred to was largely confirmed in the minutest details, but in looking over the results of the quarter it occurred to me that enough additional observations had been made to justify this somewhat informal record. It would be strange if the examination of large series of well made preparations by seven or eight trained observers did not result in something noteworthy, especially in a group so little studied. The drawings of the accompanying plate have been made by each student from whose work some observation has been taken, and whose notes furnish the substance of my comment. All the figures were drawn with a $\frac{1}{12}$ immersion and an Abbé camera lucida, excepting *fig. 6*, which was drawn with a $\frac{1}{6}$ dry lens.

Figure 1 is contributed by Mr. J. H. Schaffner from his work upon *Pinus Banksiana*. It is stained with cyanin-erythrosin, and represents the upper part of the oosphere, into which a pollen-tube with two nuclei is penetrating, having passed through the neck of the archegonium. The two nuclei within the tube are those of the male cells, which were differentiated within the pollen grain, the difference in size







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having been developed since entering the oosphere. In the case of *P. silvestris*, Dixon has observed that the tube nucleus and the stalk-cell nucleus may accompany the two male cells into the oosphere, but in this case no trace of these sterile nuclei could be found, and before the tube began to enter the oosphere they had given evidence of the beginning of disorganization. The most remarkable feature of the section, however, is the bulging of the female nucleus (F) towards the larger and nearer male nucleus (M). Mr. Schaffner has observed[1] a similar bulging of the oosphere nucleus in *Alisma*, but in that case the whole side of the nucleus appeared to be drawn out, while in the case before us there is only a papilla-like protuberance.

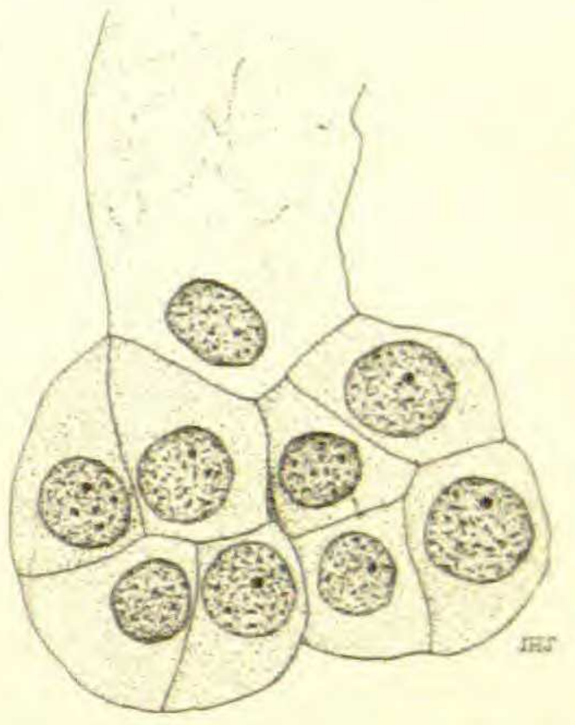

FIG. 1.—Two embryos with a single suspensor

Figure 2 is contributed by Mr. Charles J. Chamberlain, and is a fitting supplement to the stage found by Mr. Schaffner, although it was obtained from another species, *P. Laricio*, the common Austrian pine of the parks. The male (M) and female (F) nuclei are in the initial stage of fusion, the protuberance of the latter having decidedly indented the former. In 2A Mr. Chamberlain has outlined the embryo-sac in order to locate the pairing nuclei, which are nearer the micropylar end. It will be observed, therefore, that the male nucleus is not upon the side of its entrance, a shift in position which may be common, or it may be the accident of the section. The male nucleus also has increased in size until it approximates that of the female nucleus, an increase that seems to begin in the case of one of the two male nuclei when they enter the oosphere, as shown in *fig. 1*. In each nucleus the nucleolus (N) has broken up into numerous globules. In both cases, also, the chromatin filament shows plainly, and is probably in one continuous piece, the free ends being due to cutting, since the nuclei appear in three sections of the series. This state was discovered before that represented by *fig. 1*, and the sexual nature of the two nuclei was much in doubt. The micropylar position and the protuberance of the one seemed to argue for its male character, but the smaller size of the other was against its female character. As the preparation had been stained for ordinary class use it was a question whether sexual staining would be possible. The cover was removed,

[1] BOT. GAZ. 21: 127. 1896.

and an attempt was made to restain with cyanin-erythrosin, but the nuclei still stained alike. Dr. Watasé's researches on the sexual nuclei of animals show that at the moment of fusion the nuclei stain alike, while before fusion the male nucleus is cyanophilous and the female nucleus erythrophilous. However, the present attempt at sexual staining proves nothing, as the sections threatened to wash off, and consequently the staining was not prolonged enough to become decisive.

Figure 3 is contributed by Mr. John G. Coulter, and represents a young embryo of *Pinus Laricio* that has developed at the end of two, and probably four, suspensors. The general statement that in *Pinus* each of the four independent suspensors develops an embryo breaks down in this species. The statement usually runs that *Picea excelsa* is the single exception among the *Abietinæ*. In *Pinus Laricio*, however, the greatest variety was observed; sometimes an embryo to each suspensor; oftener an embryo to two or four suspensors, as in the figure; and in one case two embryos to a single suspensor, as shown in the accompanying cut, furnished by Mr. Schaffner. In the last case the primary segmentation was evidently longitudinal, the two resulting cells for some reason became physiologically dissociated, and each one of them proceeded independently to form an embryo.

Figure 4 is contributed by Mr. W. D. Merrell, and represents the first segmentation of an embryo of *Pinus Banksiana*. In this species it seems to be the rule for the first one or two segmentations to be transverse. Afterwards longitudinal divisions appear, beginning with the basal cell and including the apical cell. In *P. Laricio* the primary segmentation is also usually transverse, the only exception noted being that represented in the text cut, and there is that general freedom from any fixed order in the subsequent segmentations which Strasburger figures for *Thuja*. Nothing that could be regarded as a true apical cell was observed in any case, for though the form of an apical cell was simulated occasionally, its subsequent history showed that it was a resemblance in form and not in fact, for it never cut off successive oblique segments, or even one.

Figure 5 is contributed by Mr. O. W. Caldwell, and represents the tip of a pollen-tube of *Pinus Laricio* after it has passed through the nucellus and is in the immediate neighborhood of the archegonia. The tip is considerably swollen, as if the protoplasmic contents of the tube are being crowded into it. The four nuclei are plainly seen, as